



Factors Associated with First Service Conception Rate in Dansk Holstein Cows Raised on a Farm in Southern Denmark

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ABSTRACT

The objective of this study was to investigate the effects of different factors on the first service conception rate (CR) in Dansk Holstein cows raised on a farm in the Southern Denmark. Data including age of cows, parity, age at the first service (AFS), age at the first calving (AFC), calf's gender, previous gestation length, calving to service interval (CSI), calving season, and service season of 699 cows was collected from the herd's database. Logistic regression was used to evaluate the effects of the investigated factors on the CR. Results showed that AFS, AFC, calving interval, CSI, and CR were 410.6±22.2, 714.5±50.2, 394.5±59.7, 87.6±23.7 days, and 40.8%, respectively. The odds of conception in parity 3 (OR=0.41, 95%CI=0.26-0.64, P<0.001) was lower than that in parity 1. A CSI of 36-60 days (OR=0.28, 95%CI=0.16-0.48, P<0.001) and 60-80 days (OR=0.50, 95%CI=0.29-0.85, P<0.01) resulted in a lower CR than a CSI of 81-90 days. Cows calving in spring (OR=3.59, 95%CI=1.76-7.33, P<0.01), summer (OR=3.24, 95%CI=1.64-6.42, P<0.01), and winter (OR=3.89, 95%CI=2.07-7.30, P<0.001) had a higher CR than in autumn. Cows having service in spring (OR=3.49, 95%CI=2.16-5.63, P<0.001), summer (OR=3.29, 95%CI=2.08-5.19, P<0.001), and autumn (OR=3.79, 95%CI=2.23-6.43, P<0.001) had a higher CR than in winter. An AFC of 671-700, 701-730, and >760 days (OR=0.34-0.52, P<0.05) conferred a lower CR than that of 646-670 days. This study indicates that parity >2, AFC >670 days, CSI <60 days, calving in autumn, and service in winter are risk factors for reduced CR in the investigated Dansk Holstein cows.

Key words: Age, Conception rate, Holstein cows, Reproduction, Season.

INTRODUCTION

First service conception rate (CR) is one of the most important reproductive parameters in dairy production. Recent studies reported that the CR in dairy cows varied between 31.0 and 52.4% (Borş et al. 2025; Castillo et al. 2025; Garnsworthy et al. 2025). A high CR results in a short calving interval, decreases the culling rate and insemination cost, and increases the milk production, therefore increases the farm profit (Sasaki et al. 2019). However, in recent decades, while milk yield has been increased due to the improved genetic selection and nutrition management the CR has been reduced due to increased rates in early embryonic death (Diskin et al. 2006). Knowing the factors that contribute to the CR in dairy cows will help diminish their harmful effects and improve their beneficial effects, subsequently improving the CR.

The CR may be affected by several factors including intrinsic cow's factors and extrinsic factors such as environment and management (Borş et al. 2025; Ojo et al. 2025; Pérez-Reboloso et al. 2025). Of these factors, parity, age, calving to the first service interval, and season of calving/service are commonly investigated with controversial results. Parity is commonly known to have a negative association with CR (Inchaisri et al. 2010; Irikura et al. 2018b; Borş et al. 2025) but its non-significant effects were also reported by others (Buckley et al. 2003; Souames and Berrama 2020). Among the studies that found significant effects, some witnessed a dramatic decrease in CR when parity increased (Balendran et al. 2008), others found a gradual decrease (Inchaisri et al. 2010; Irikura et al. 2018b; Borş et al. 2025). Calving to the first service interval (CSI) is positively associated with CR (Buckley et al. 2003; Sasaki et al. 2016; Irikura et al. 2018b; Filho et al. 2022)

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but the most fertile CSI has not been determined. Irikura et al. (2018b) reported that CR was similar among CSI of 49-64, 64-89 and ≥ 90 days, while Filho et al. (2022) found that a CSI of 50-59 days conferred a lower CR in comparison with that of ≥ 80 days. The association between season of service and CR is also inconsistent. In Poland and Algeria, CR was higher in winter than in summer (Siatka et al. 2017; Souames and Berrama 2020). By contrast, in Norway, CR was higher in summer than in winter (Refsdal 2007). In Romania, CR was higher in spring than in summer and autumn (Borş et al. 2025). In Japan, the same groups of authors reported different effects of season of service on CR in the same cows breed (Irikura et al. 2018a; Irikura et al. 2018b). Given such inconsistent results, further studies are necessary to provide more understandings of the effects of different factors on CR in cows.

The present study was aimed at investigating the effects of age at the first service, age at the first calving, parity, age, previous gestation length, gender of the calf, calving to the first service interval, season of calving, and season of service on the first service conception rate of Dansk Holstein cows raised on one farm in Denmark.

MATERIALS AND METHODS

Ethical approval

This research strictly followed ethical standards, involving no sample collection, thereby eliminating the need for specific ethical approval while ensuring compliance with animal welfare regulations.

Study animals

This study was conducted on Holdan Argo farm in Tyrholmvej 7, 6230 Rødekro, Denmark. Located in the Southwest of Denmark, the farm had about 800 milking Dansk Holstein dairy cows. All the cows included in the study were born between 25th January 2017 and 15th September 2021 and were in parities 1-4 averaging 1.8. Cows were kept indoor year round and the barn was lit 16 hours per day. They were milked twice per day with the first milking starting at 3AM until 11AM and second milking starting at 3PM until 10:30PM. The milking cycle was about 320 days and dry off period was about 38-43 days. The average milk production was 11600kg per milking cycle. Heat was detected by using the Nedap heat detection system. In-heat cows were artificially inseminated with frozen-thawed semen once per estrus. Most of the heifers were inseminated with sexed semen. In the heifers, pregnancy examination was conducted using either rectal palpation or ultrasound on day 35 after insemination by skillful veterinarians. In the cows, the pregnancy examination was not conducted, cows that did not exhibit estrus within 45 days after insemination were considered pregnant. Cows were vaccinated against Rotavirus, Coronavirus, and *E. coli* before the dry off period to prevent the scour in the calves (Bovigen® Scour, Virbac). In total, data of 699 cows were collected.

Data collection

Data was collected from the Dairy Management System stored in a computer. Data including cow identification, date of birth of the cows, date of the first service, date of the first calving, date of the service that

resulted in pregnancy of the recent calving, date of the recent calving, date of the first service after the recent calving, sex of the calves in the recent calving and conception status of the cows were collected. From the collected information, AFS (day), AFC (day), previous gestation length (day), CSI (day), and CI (day) were calculated. The average CI was calculated based on the data of 573 CI in 415 cows in the parity 2-4. The standard deviation of CI, however, was calculated based on 265 CI of 265 cows in parity 2 due to the lack of information on individual CI in parity 3-4 cows. The last date of data collection was 17th December 2023 and the last service was conducted on 2nd November, 2023.

Previous gestation length was partitioned into 3 groups, i.e., 244-275, 276-280, and 281-292 days. AFS was partitioned into 3 groups including 365-400, 401-430, and >430 days. AFC was partitioned into 5 groups including 646-670, 671-700, 701-730, 731-760, and >760 days. Age of the cows was partitioned into 4 groups including 730-1095, 1096-1460, 1461-1825, and >1825 days. Months were grouped into seasons, i.e., Winter (December - March), Spring (April - May), Summer (June - August), and Autumn (September - November). The average monthly temperature spanned from 1.96 to 4.75°C in winter, 8.99 to 13.16°C in spring, 16.39 to 18.45°C in summer, and 6.56 to 14.99°C in autumn. CSI was partitioned into 5 groups, i.e., 36-60, 61-80, 81-90, 91-100, and 101-209 days.

Statistical analysis

Descriptive statistics including mean, standard deviation, and percentage were used for description of the data. Logistic regression was used to analyze factors associated with CR. The dependent variable was CR (yes/no) resulted from the first service after the recent calving and the independent variables were parity, gestation length, gender of the calf, AFS, AFC, CSI, season of calving, and season of service. At first, univariate analyses were conducted to observe the effect of each single factor on the CR. After that multivariate analyses were conducted using a forward pattern to build the multivariate models containing factors that most significantly affected the conception rate at first service post-calving. Due to a strong association between season of service and season of calving (Spearman's rho correlation coefficient = 0.822), these two independent factors were analyzed in two separate multivariate models. All analyses were conducted in the Statistical Package for the Social Sciences (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). A P value less than 0.05 was considered significant.

RESULTS

The descriptive statistics of the investigated cows are presented in the Table 1. The average AFS and AFC were 410.6 and 714.5 days, respectively. The CSI and CI were 87.6 and 394.5 days, respectively. The CR was 40.8% (285/699).

Univariate logistic regression showed that parity, age of the cows, gender of the calf, AFC, CSI, season of calving and season of service had significant effects on conception rate. By contrast, previous gestation length and AFS did not influence conception rate (Table 2).

Table 1: Descriptive statistics of 699 Dansk Holstein cows

Parameters	Mean±SD
Parity	1.8±0.9
Age at the first service (day)	410.6±22.2
Age at the first calving (day)	714.5±50.2
Recent gestation length (day)	277.3±4.9
Calving to the first service interval (day)	87.6±23.7
Calving interval (day)*	394.5±59.7
Conception rate at first service post-calving (%)	40.8

Data are presented as mean and standard deviation. *The mean value of calving interval was calculated from 573 calving intervals in 415 cows in parities 2-4, and its standard deviation was calculated from 265 calving intervals of 265 cows in the parity 2.

Two multivariate models that contained the most significant factors for CR were built (Table 3). The first model selected parity, AFC, and season of service, and the second one consisted of AFC, season of calving, and CSI. The first model explained 14.3%, and the second model explained 11.1% the variation of the conception rate. In case age of the cows was replaced by parity in the first

model, the model explained 13.7% the variation of the CR. The first multivariate model showed that parity 3 cows had a lower odd of being conceived (OR=0.41, 95% CI=0.26-0.64, $P<0.001$) than parity 1 cows. Cows that were serviced in spring, summer, or autumn had significantly higher conception rates compared to those serviced in winter, with ORs of 3.49 (95% CI: 2.16–5.63), 3.29 (95% CI: 2.08–5.19), and 3.79 (95% CI: 2.23–6.43), respectively ($P<0.001$). The second multivariate model showed that cows that had a CSI of 36-60 days (OR=0.28, 95% CI=0.16-0.48, $P<0.001$) and 60-80 days (OR=0.50, 95% CI=0.29-0.85, $P<0.01$) had a lower odd of being conceived in comparison with cows that had a CSI of 81-90 days. Cow that calved in spring (OR=3.59, 95% CI=1.76-7.33, $P<0.01$), summer (OR=3.24, 95% CI=1.64-6.42, $P<0.01$), and winter (OR=3.89, 95% CI=2.07-7.30, $P<0.001$) had higher odds of being conceived than cows calved in autumn. Cows that had an AFC of 671-700, 701-730, and >760 days had a lower odd of being conceived (OR=0.34-0.52, $P<0.05$) than cows had an AFC of 646-670 days.

Table 2: Univariate analysis of factors associated with conception rate at first service post-calving in 699 investigated Dansk Holstein cows

Investigated parameters	Conception rate (%)	OR (95% CI)
Parity		
1 (n=284)	48.2	1
2 (n=265)	41.9	0.77 (0.55-1.08)
3 (n=142)	25.4	0.36 (0.23-0.57)***
4 (n=8)	12.5	0.15 (0.02-1.26)
Age (days)		
730-1095 (n=282)	48.2	1
1096-1460 (n=272)	40.8	0.74 (0.53-1.04)
1461-1825 (n=138)	26.8	0.39 (0.25-0.61)***
1826-2312 (n=7)	14.3	0.18 (0.02-1.51)
Previous gestation length (days)		
244-275 (n=216)	39.8	1
276-280 (n=316)	41.1	1.06 (0.74-1.50)
281-292 (n=167)	41.3	1.06 (0.71-1.61)
Gender of the calf		
Heifer (n=466)	43.8	1
Bull (n=233)	34.8	0.68 (0.49-0.95)*
Age at first artificial insemination (days)		
365-400 (n=219)	44.7	1
400-430 (n=387)	39.3	0.79 (0.57-1.11)
>430 (n=93)	37.6	0.74 (0.45-1.22)
Age (days) at first calving		
646 -670 (n=66)	53.0	1
671-700 (n=270)	43.0	0.69 (0.40-1.18)
701-730 (n=194)	35.6	0.50 (0.29-0.89)
731-760 (n=85)	47.1	0.81 (0.42-1.55)
>760 days (n=84)	29.8	0.39 (0.20-0.76)**
Calving to the first service interval (days)		
36-60 (n=109)	22.0	0.28 (0.16-0.48)***
61-80 (n=96)	33.3	0.50 (0.30-0.83)**
81-90 (n=183)	50.3	1
91-100 (n=167)	44.3	0.79 (0.52-1.20)
100-209 (n=144)	43.8	0.77 (0.50-1.19)
Season of calving		
Winter (December-March) (n=377)	44.6	3.43 (1.89-6.24)***
Spring (April-May) (n=104)	42.3	3.13 (1.68-6.20)**
Summer (June- August) (n=139)	41.7	3.06 (1.59-5.89)**
Autumn (September - November) (n=79)	19.0	1
Season of service		
Winter (December-March) (n=189)	19.6	1
Spring (April-May) (n=176)	48.9	3.93 (2.47-7.25)***
Summer (June- August) (n=221)	46.6	3.59 (2.30-5.60)***
Autumn (September - November) (n=113)	52.2	4.49 (2.68-7.51)***

OR: odds ratio, CI: confidence interval, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Table 3: Multivariate analysis of factors associated with conception rate at first service post-calving in 699 investigated Dansk Holstein cows

Investigated parameters	Model 1; OR (95%CI)	Model 2; OR (95%CI)
Parity		
1	1	
2	0.76 (0.53-1.10)	
3	0.41 (0.26-0.64)***	
4	0.25 (0.03-2.14)	
Age (days) at first calving		
646 -670	1	1
671-700	0.59 (0.33-1.06)	0.52 (0.29-0.94)*
701-730	0.45 (0.24-0.85)*	0.37 (0.20-0.68)**
731-760	0.67 (0.33-1.34)	0.69 (0.34-1.37)
>760	0.34 (0.17-0.71)**	0.34 (0.16-0.70)**
Season of service		
Winter (December-March)	1	
Spring (April-May)	3.49 (2.16-5.63)***	
Summer (June- August)	3.29 (2.08-5.19)***	
Autumn (September - November)	3.79 (2.23-6.43)***	
Season of calving		
Winter (December-March)		3.89 (2.07-7.30)***
Spring (April-May)		3.59 (1.76-7.33)**
Summer (June- August)		3.24 (1.64-6.42)**
Autumn (September - November)		1
Calving to the first service interval (days)		
36-60		0.28 (0.16-0.48)***
61-80		0.50 (0.29-0.85)***
81-90		1
91-100		0.71 (0.46-1.10)
100-209		0.73 (0.46-1.15)

OR: odds ratio, CI: confidence interval, *P<0.05, **P<0.01, ***P<0.001.

DISCUSSION

In this study, the factors associated with the first service conception rate at the first service post calving were investigated in Dansk Holstein cows raised in one farm in the Southwest of Denmark. The results indicate that parity, age at the first calving, calving to the first service interval, and seasons of calving/service significantly affected the first service conception rate.

Previous study showed that the AFC of Holstein cows were 728 days in Czech Republic (Krpálková et al. 2014), 737 days in the United States of America (Hutchison et al. 2017), 760-805 days in Iran (Nilforooshan and Edriss 2004; Atashi and Hostens 2021) and 783 days in Italy (Ferrari et al. 2024), which are higher than the AFC found in this study. The CSI in this study, however, falls in the range of the results reported by previous studies which varied from 67 to 91.2 days (Refsdal 2007; Ansari-Lari et al. 2010; Sasaki et al. 2016; Nabenishi and Yamazaki 2017; Kim and Jeong 2019). The CI of the investigated cows is equal to 391.3 days reported by Ojo et al. (2025) and 392.9 days which was reported by Irikura et al. (2018b), but shorter than 399-438 days which was reported by other authors (Ansari-Lari et al. 2010; Krpálková et al. 2014; Hutchison et al. 2017; Arroyo-Rebollar and López-Villalobos 2021; Ogawa and Satoh 2021). The 40.8% first-service conception rate observed in this study aligns with the rates of 41.6–42.3% reported in previous studies (Ansari-Lari et al. 2010; Kim and Jeong 2019), but is lower than the higher rates of 44.7–53.5% documented in other research (Souames and Berrama 2020; Temesgen and Assen 2022; Condon et al. 2024; Borş et al. 2025; Castillo et al. 2025). However, the CR observed in this

study was higher than the 31% reported in a recent study (Garnsworthy et al. 2025).

The lower CR in the parities 3-4th cows in comparison with that in the parities 1-2 supports findings of some other previous studies (Balendran et al. 2008; Inchaisri et al. 2010). The CR of the first parity Holstein cows was higher than that in 2nd and 3-4th parity cows (42.9% vs. 20.9 and 11.9%, respectively) (Balendran et al. 2008). Similarly, CR in 1st parity Dutch dairy cows was 43% which was higher than that in other parities (32-39%) (Inchaisri et al. 2010). Other authors found that the CR in first-parity cows was 49.6%, while it was 40.2% and 37.1% in second- and third-parity cows, respectively (Borş et al. 2025). Some authors found that CR began to decrease after the parity 6th (Sasaki et al. 2016). In older cows, there is a decline in the number of granulosa cells and the volume of follicular fluid leading to the impairment of oocyte development (Iwata 2017). Furthermore, in cold environment the uterine involution is inferior in parities ≥2 cows in comparison with that in primiparous cows (El-Din Zain et al. 1995) and in parities 3-4 cows compared to that in parity 2 cows (Luís-Balarezo et al. 2018). Therefore, the decrease CR in the parities 3-4th cows might partly be due to the age-associated decline in quality of the oocytes and uterine involution. However, the reason(s) for the rapid decline in CR is yet to be identified.

In this study, almost all cows (94.9%) calved in autumn would be serviced in winter and might be suffered, in some degree, from cold stress. The lower CR in cows serviced in winter corroborated findings by many previous studies. In cold season (-38-0°C) the CR at 60 days post service was 35.0% compared to 66.6% in the warm season (0-32°C) (Cengiz et al. 2022). Similarly, in cold stress condition (<4°C) cows had 0.82 lower odds of conception

in comparison with cows inseminated in no stress condition (4-29°C) (Chebel et al. 2007). Other authors also found a reduced CR in winter (Sasaki et al. 2016; Nabenishi and Yamazaki 2017). Cold stress increases energy requirement in the cows (Huang et al. 2025). However, under this condition, the digestive function of the cows is decreased simultaneously with increased energy metabolism and stress hormone imbalance leading to negative energy balance (Wang et al. 2023). In turn, negative energy balance decreases the circulating insulin like growth factor-1, the follicular growth rate and the quality of the oocytes, and increases the embryonic mortality rate (Fenwick et al. 2008; Song et al. 2021; Wang et al. 2023; Serbetci et al. 2024). These conditions may explain the lower CR in cows calved in the autumn or in cows serviced in winter in this study.

Previous studies found that there was a positive association between CR and CSI (Buckley et al. 2003; Sasaki et al. 2016; Irikura et al. 2018b; Filho et al. 2022). When CSI increased from less than 40 days to more than 80 days the CF of Brazilian cows increased from below 40% to above 60% (Filho et al. 2022). Similarly, when CSI increased from below 48 days to over 90 days the odds of being pregnant in Japanese Black cattle increased from 0.46 to 1 (Irikura et al. 2018b). In another study, the number of services per conception in Japanese Black cattle was highest when CSI was below 50 days, and lowest when the CSI was over 95 days (Sasaki et al. 2016). In this study, the lowest CR in cows with CSI of less than 60 days and the increase in CR when the CSI increased might be due to some reasons. First, the uterine involution completes at about 49 days postpartum (Scully et al. 2013) and in some cows this duration is longer. Second, the prevalence of subclinical endometritis decreases over time postpartum, and from 8-28% of cows may still suffer from subclinical endometritis at ≥ 7 weeks after calving (Arias et al. 2018). Third, the prevalence of cows that attains positive energy balance increased over time after calving, and only 92% of primiparous cows and 73% of multiparous cows reach this condition at 49 days postpartum (Grummer and Rastani 2003). Collectively, when the CSI increases the harmful effects of incomplete uterine involution, subclinical endometritis, negative energy balance on reproductive performance decreases. Therefore, CR in the cows with CSI of 81-90 was higher than that in the cows with CFSI of 36-60 and 61-80 days.

In this study, an AFC of 646-670 days or 21.5-22.3 months increased the odds of being conceived at the first service compared with a higher AFC. An insufficient number of studies have assessed the effect of AFC on the CR in subsequent parities in Holstein cows. A scarce study investigated the effect of AFC on reproductive performance in Holstein cows found that lower AFC resulted in shorter calving to conception intervals (Recce et al. 2021). Rather, previous studies mainly focused on investigating the effect of AFC on productive performance. AFC in Holstein cows has been suggested to be lower than 22.5 months to increase survivability and lifetime milk yield without impairment of reproductive performance (Kusaka et al. 2023). Other studies suggested that optimal AFC should be as low as 24 months (Nilforooshan and Edriss 2004) or 22.5-23.5 months (Do et al. 2013) to increase lifetime profit. The result of this study suggested

that AFC of Dansk Holstein cows could be reduced to 21.5-22.3 months to increase the CR in the subsequent parities, and the effects of a short AFC on other reproductive parameters are in the need of investigation.

Conclusion

This retrospective study indicates that the most significant factors affecting first service conception rate in the investigated Dansk Holstein cows are parity, age at first calving, calving to service interval, and seasons of calving and service. The age at first calving >22.3 months, parity ≥ 3 , calving to service interval <80 days, calving in autumn, and service in winter contribute to the decrease in conception rate of the investigated animals.

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Author's Contribution: Nguyen Hoai Nam: Conceptualization, Data analysis, Original draft preparation; Le Thi Le Thuong: Conceptualization, Data collection; Do Thi Kim Lanh: Conceptualization, Reviewing-Editing; Bui Van Dung: Writing-Reviewing-Editing; Peerapol Sukon: Conceptualization-Reviewing-Editing.

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